Product information

User's Manual

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IgG_4 Screen Nutritional 20 ELISA

Enzyme immunoassay for the detection and the quantitative determination of specific IgG4 antibodies against food antigens in serum and plasma

CE

IVD

REF **DE40496**

Σ **96 wells**

Version 04b-03/14
JS
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1. INTENDED USE

The Demeditec IgG4 Screen Nutritional 20 ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgG_4 antibodies against food antigens in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Demeditec. Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

2. GENERAL INFORMATION

Incompatibility reactions against food may cause various symptoms in the human organism and this disturbance is manifested in the immune system by the formation of specific IgE, IgG or IgG_4 antibodies. Statistics show that 60% of the population suffer from intolerances against at least one foodstuff, which may cause clinical symptoms or enhance them. Hints may be various and reach from skin irritations over digestive disorders up to migraine. With the diagnostic findings of unspecific discomfort, allergies or intolerances against food should be clarified. The theoretical basis for the determination of specific IgG or IgG_4 for the diagnosis of food intolerances depends on the observation that some subclasses of IgG (mainly IgG_4) are connected to the in vitro degranulation of basophilic cells and mastocytes and the activation of the complement cascade. It was also observed that high concentrations of circulating IgG were measured in atopic persons. Already early surveys showed that in persons with inflammatory reactions against food IgG but not IgE was detected. Significantly enhanced IgG and IgG_4 titers were also found in patients with food intolerances. Skin tests are relatively poorly correlated to food allergies and are only significant in the presence of IgE related reactions. As additional diagnostic tools provocation and elimination diets are applied. These methods depend strongly on the motivation and compliance of the patient. Due to these constraints nowadays serological determinations of antibodies against various food panels are applied increasingly. The two reactions related with the immune system differ insofar as the IgE associated food allergy occurs within the next hour following the food intake, while IgG/IgG_4 intolerances show a delayed reaction of 24 to 120 hours and persistent symptoms may arise.

3. PRINCIPLE OF THE TESTS

The Demeditec IgG4 Screen Nutritional 20 ELISA test kit is based on the principle of the enzyme immunoassay (EIA). With each test kit 4 patients can be tested. 20 different food antigens and 4x reference antigen (egg white, f01) for standards are bound on the surface of three colour-coded microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG_4 antibodies of the serum and the immobilized antigens takes place. After a one hour incubation at 37°C, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG_4-AP conjugate is added and incubated for 30 minutes at 37°C. After a further washing step, the substrate (PNPP) solution is pipetted and incubated for 60 minutes at 37°C, inducing the development of a yellow dye in the wells. The color development is terminated by the addition of a stop solution. The resulting dye is measured spectrophotometrically at the wavelength of 405 nm. The concentration of the IgG_4 antibodies is directly proportional to the intensity of the color.

4. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25°C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

5. REAGENTS PROVIDED

The IgG_4 Screen Nutritional 20 ELISA kit contains sufficient reagents for 4 patients (20 determinations each). The first strip of the plate contains reference antigen (egg white, f01) for the generation of a calibration curve.

Symbol	Components	Volume / Qty.
SORB MT	Food/Reference antigen coated microtiter plate	1
CAL A – D	Standards: 0.35, 0.70, 3.5, 17.5 U/mL	0.5 mL each
ENZ CONJ	Anti-human IgG_4 Enzyme Conjugate	15 mL
SUB PNPP	Substrate	15 mL
STOP SOLN	Stop Solution	15 mL
SAM DIL	Sample Diluent	40 mL
WASH SOLN 10x	Washing Buffer (10×)	60 mL

Storage and Stability (refer to the expiry date on the outer box label)
Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.

5.1. SORB MT Microtiter Plate
1 microtiter plate à 96 wells for 4 patients. Three color-coded microtiter strips (green, yellow, red) with 8 wells each, coated with 20 food antigens and 4x reference antigen. See distribution scheme. Ready-to-use.

5.2. CAL A – D Standards A - D
0.5 mL each, human plasma diluted with PBS/BSA, with 0.35, 0.70, 3.5, and 17.5 U/mL of IgG_4 antibodies against egg white (f1). Addition of 0.05% sodium azide. Ready-to-use.

5.3. ENZ CONJ Anti-human-IgG_4 Enzyme Conjugate
15 mL, mouse-a-human-IgG_4-AP, in proteinacious buffer solution. Addition of 0.01% methylisothiazolone, 0.01% bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

5.4. SUB PNPP Substrate
15 mL, PNPP (Paranitrophenylphosphate). Ready-to-use.

5.5. STOP SOLN Stop Solution
15 mL, 1 M sodium hydroxide. Ready-to-use.

5.6. SAM DIL Sample Diluent
40 mL, PBS/BSA buffer. Addition of 0.05% sodium azide. Ready-to-use.

5.7. WASH SOLN 10x Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

6. MATERIALS REQUIRED BUT NOT PROVIDED

- 30 µL, 100 µL and 1000 µL micro- and multichannel pipettes
- Microtiter Plate Reader (405 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Deionized water
- Re-usable black lid for covering (Available upon request at Demeditec Diagnostics GmbH)

7. SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 7 days. For a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.
For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 30 µL serum + 3 mL sample diluent).

8. ASSAY PROCEDURE

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

8.2. Assay Steps

1. For each patient sample prepare three microtiter strips (order: green, yellow, red).
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards (first 4 wells of the green strip) respectively into the wells (see distribution scheme).
3. Cover plate with the re-usable plate cover and incubate for 60 minutes at 37°C.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells.
6. Cover plate with the re-usable plate cover and incubate for 30 minutes at 37°C.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells.
9. Cover plate with the re-usable plate cover and incubate for 60 minutes at 37°C.
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 405 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

9. EVALUATION

The evaluation can be performed either in units per mL (U/mL) or in classes.

Example

Standard	Class	OD-Value
0.35 U/mL	1	0.145
0.7 U/mL	2	0.250
3.5 U/mL	3	0.620
17.5 U/mL	4	1.715

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

Quantitative Evaluation
The ready-to-use standards and controls of the IgG_4 Screen Nutritional 20 ELISA test kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. The values for controls and standards in units are printed on the labels of the vials. For a quantitative evaluation the absorptions of the standards are graphically drawn *point-to-point* against their concentrations. From the resulting reference curve the concentration values or the respective reaction class for controls and each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit *point-to-point* has to be chosen.

10. ASSAY CHARACTERISTICS

Spez. IgG_4 ELISA	**Egg White**	**Cow's Milk**	**Tomato**
Intra-Assay-Precision	7.7 %	8.0 %	8.7 %
Inter-Assay-Precision	6.6 – 10.9 %	8.4 – 13.0 %	4.6 – 7.4 %
Inter-Lot-Precision	2.5 – 11.4 %	5.6 – 11.8 %	0.5 – 9.6 %
Analytical Sensitivity	0.22 U/mL	0.17 U/mL	0.16 U/mL
Recovery	90 – 107 %	89 – 103 %	87 – 97 %
Linearity	82 – 114 %	73 – 100 %	102 – 120 %
Cross-Reactivity	No cross reactivity towards IgE up to 100000 IU/mL.		
Interferences	No interferences with bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.		
Clinical Specificity	88 %	86 %	90 %
Clinical Sensitivity	86 %	94 %	80 %

11. REFERENCES

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SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore